

# Supporting Information

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## SI Results and Discussion

$^{15}\text{NH}_4^+$  oxidation rates were lower overall in the HOT 210 experiment compared with HOT 209, and in accordance with the findings of Sutka et al. (1) and Dore and Karl (2), differences in rate profiles between the two cruises suggest variability in the magnitude and distribution of nitrification rates over time at station ALOHA. Specifically, HOT 210 exhibited a broad rate maximum extending from 150 to 300 m where rates ranged from 2.63 to 2.89  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ , whereas HOT 209 rates peaked at 10.3  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 150 m (Fig. S5). These rates are higher than those reported from 145, 155, and 165 m at Station ALOHA by Sutka et al. (1); these authors measured a maximum oxidation rate of 1  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  in May 2000, using additions of  $^{15}\text{NH}_4^+$  label.

Dore and Karl (2) reported rates ranging from 1 to 137  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  during three HOT cruises to Station ALOHA. Rates ranged from 10  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 131 m to 137  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 152 m during HOT 31 (October 1991), from 1  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 140 m to 80  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 172 m during HOT 36 (April 1992), and from 1  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 106 m to 23  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  at 155 m during HOT 50 (October 1993). These measurements differ from our approach in that they were made using  $^{14}\text{C}$ - and chemical-based techniques, but assuming that they yield comparable numbers to the more direct  $^{15}\text{NH}_4^+$  technique we used, they are clearly indicative of variability in ammonia oxidation rates at Station ALOHA. The maximum rate in these studies and our experiments was found at the base of the euphotic zone over a similar range of depths, but the rate ranged over two orders of magnitude, from 1  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  (1) to 2.89–10.3  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  (this study) to 23–137  $\text{nmol}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$  (2).

We found similar temporal variability in the response of ammonia oxidation rates to pH changes  $>0.14$  at Station ALOHA: In the HOT 209 experiment,  $^{15}\text{NH}_4^+$  oxidation rates decreased monotonically with decreasing pH values, whereas this response was not observed during HOT 210 (Table S2). Rates significantly decreased during HOT 210 when  $\text{pH}_{\text{total}}$  was reduced from 7.99 to 7.85, but oxidation rates measured at pH 7.63 and 7.42 were not significantly different from those measured at higher pH or from each other. The greatest interreplicate variability was in fact measured at pH 7.42—coefficients of variation were 11–21% (Table S2). A  $\text{pH}_{\text{total}}$  change of 7.99–7.42 was included as an extreme end member, however, as it represents a nearly fourfold increase in  $[\text{H}^+]$  and  $p\text{CO}_2$  of  $\sim 1,500$   $\mu\text{atm}$ —these values will not be observed anywhere other than in the deep ocean (e.g., below 700 m at station ALOHA, ref. 3) for more than a century. However, in coastal waters this level of pH change produced considerable variation in ammonia oxidation rates between and within experiments (4); over multiyear timescales, Rudd et al. (5) observed a threshold effect in experimentally acidified lakes, where a 0.1–0.3 pH decrease reduced ammonia oxidation by 14–16% in different lakes, but a change of 0.6–0.8 reduced rates by  $>92$ –96%. Taken together, these findings indicate that certain ammonia oxidizer communities—present at different times or in different locations—may be more or less sensitive to pH change of  $>0.14$ . For instance, AOA may be less sensitive to pH than AOB (6), or particular clades of AOA or AOB may be more or less sensitive to pH change.

## SI Materials and Methods

**Sample Collection.** Water samples for Sargasso Sea experiment 1 were collected May 17, 2008 at 240 m at 20°26.62' N, 45°52.72' W; samples for Sargasso Sea experiment 2 were collected at 240 m on May 19, 2008 at 23°35.19' N, 50°54.63' W; samples for the

BATS experiment were collected at 150 m on May 24, 2008 at 31°39.63' N, 64°9.7' W; samples for the SPOT experiment were collected at 45 m on June 18, 2008 at 33.33°N, 118.24°W. Water samples for both HOT experiments were collected at 175 m at 22°45'N, 158°00'W on Feb 18, 2009 (HOT 209) and April 28, 2009 (HOT 210).

**Experimental Details.** In the Sargasso Sea, BATS, and SPOT experiments, 2-L amber plastic bottles (Sargasso and BATS) or 20-L carboys (SPOT) filled with seawater were manipulated by gentle bubbling via plastic diffusers at uniform rates with high efficiency particulate air (HEPA)-filtered, commercially prepared, air:CO<sub>2</sub> mixtures (Scott Gas). All treatments were manipulated with air:CO<sub>2</sub> mixtures in the Sargasso Sea, whereas controls consisted of  $^{15}\text{NH}_4^+$ -amended but otherwise untreated seawater at BATS and SPOT. Bottle caps were customized for gas inlets/outlets and were connected to air:CO<sub>2</sub> mixtures using acid-washed tubing. Sargasso Sea and BATS experiments were equilibrated for 4 h, distributed in 2-L amber plastic bottles, sealed for 6–14 h, and maintained in a laboratory incubator at in situ temperature (Table 1). SPOT experiments were altered with CO<sub>2</sub> for 5 d in a dark, constant temperature room (12 °C). HOT experiments were performed in 1-L amber plastic bottles that were sealed and submerged in surface seawater after pH was adjusted by addition of 0.05 M trace-metal grade hydrochloric acid (HCl). All treatments in all experiments were performed in triplicate.

**Carbonate System Parameters.** Seawater CO<sub>2</sub> parameters were verified by measurement of two carbonate system parameters: total alkalinity (TA) and dissolved inorganic carbon concentration (DIC) in the Sargasso Sea and at BATS and pH and DIC at SPOT and HOT. For the Sargasso Sea/BATS, TA was measured using a manual open-cell potentiometric TA titration system (7), whereas DIC was measured using a small-volume infrared DIC analyzer based on a LI-Cor 6262 nondispersive infrared analyzer as detector (8). Certified reference material prepared by A. Dickson at Scripps Institution of Oceanography was analyzed repeatedly to evaluate the accuracy and precision of the instruments. For the SPOT experiment, pH was measured using a combination glass electrode and DIC was measured using a conductivity flow injection analysis method (9). HOT 210 pH values were measured following incubation using m-cresol purple and spectrophotometry (3). Measured pH values were strongly correlated ( $r^2 = 0.965$ ) with expected values calculated on the basis of additions of 0.05 M HCl and archival DIC and pH data from HOT for 175 m. HOT 209 pH samples were not properly collected, but values are likely similar to HOT 210 given the correspondence between HOT 210 and archival data and that identical experimental procedures were followed for both HOT 209 and 210. DIC concentrations are measured as part of the HOT program by coulometry (3). Calculation of the full carbonate system was performed using CO2sys (10).

**$^{15}\text{NH}_4^+$  Oxidation Rate Measurements.**  $^{15}\text{NH}_4^+$  oxidation rates were measured by addition of 20–50 nM of 99 atom percent (at%) stable isotope tracer and accumulation of  $^{15}\text{N}$  label in the oxidized  $\text{NO}_2^- + \text{NO}_3^-$  pool following incubation. The  $\delta^{15}\text{N}$  value of  $\text{N}_2\text{O}$  produced from  $\text{NO}_2^- + \text{NO}_3^-$  using the “denitrifier method” (11) was measured using methods described in Popp et al. (12) and Dore et al. (13). Briefly,  $\text{N}_2\text{O}$  produced from  $\text{NO}_2^- + \text{NO}_3^-$  was stripped from a reaction vial, cryofocused (14), separated from other gases using a 0.32-mm i.d.

× 25-m PoraPLOT capillary column, and introduced into the carrier stream of a Finnigan MAT252 mass spectrometer through a modified Finnigan GC-C I interface. All  $\delta^{15}\text{N}$  values were clearly enriched (10–1,000‰) compared with in situ values of 4–7‰, which were measured to ensure the accuracy of our calculations.

Isotopic reference materials (USGS-32, NIST-3, UH  $\text{NaNO}_3$ ) bracketed every 12–16 samples and  $\delta^{15}\text{N}$  values measured on line were linearly correlated ( $r^2 = 0.996\text{--}0.999$ ) with accepted reference material  $\delta^{15}\text{N}$  values; combined  $\text{NO}_3^- + \text{NO}_2^-$  concentrations were calculated from peak areas measured on the mass spectrometer calibrated with reference materials of known concentration. Sixty-eight percent of samples were run in duplicate, and coefficients of variation for duplicate samples averaged 2.3% with a high of 7.7%. Accuracy and precision of this method were further evaluated by multiple analyses of a sodium nitrate solution for which the  $\delta^{15}\text{N}$  value of the solid  $\text{NaNO}_3$  (i.e., UH  $\text{NaNO}_3$ ) was previously determined using an on-line carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus) and were found to be  $< \pm 0.5$  ( $\pm 0.00018$  at‰  $^{15}\text{N}$ ) for samples containing  $> 2.5$  nmol of nitrate.

Initial at‰ enrichment of the substrate at the beginning of the experiment ( $n_{\text{NH}_4^+}$ , Eq. S1) was calculated by isotope mass balance on the basis of  $\text{NH}_4^+$  concentrations determined fluorimetrically (15) on frozen samples from Sargasso Sea, BATS, and HOT and within 4 h of sample collection at SPOT.  $\text{NH}_4^+$  was below the detection limit (5 nM) everywhere but SPOT, where it was 53 nM. For the isotope mass balance equation, we assumed that the  $\text{NH}_4^+$  concentration was 5 nM (with the exception of SPOT, where we used 53 nM) and that the  $^{15}\text{N}$  activity of unlabeled  $\text{NH}_4^+$  was 0.3663 at‰  $^{15}\text{N}$ .  $^{15}\text{N}$ -ammonia oxidation rates ( $^{15}R_{\text{ox}}$ ) were determined on the basis of the accumulation of  $^{15}\text{N}$  in the oxidized pool relative to the initial at‰ enrichment in the  $\text{NH}_4^+$  pool and divided by the time of the incubation. Rates were calculated using an equation modified from Ward et al. (16) and discussed by Ward and O'Mullan (17),

$$^{15}R_{\text{ox}} = \frac{(n_t - n_{\text{NO}_x^-}) \times [\text{NO}_3^- + \text{NO}_2^-]}{(n_{\text{NH}_4^+} - n_{\text{NH}_4^+}) \times t}, \quad [\text{S1}]$$

where  $n_t$  is the at‰  $^{15}\text{N}$  in the  $\text{NO}_3^- + \text{NO}_2^-$  pool measured at time  $t$ ,  $n_{\text{NO}_x^-}$  is the measured at‰  $^{15}\text{N}$  of unlabeled  $\text{NO}_3^- + \text{NO}_2^-$ ,

$n_{\text{NH}_4^+}$  is the at‰ enrichment of  $\text{NH}_4^+$  at the beginning of the experiment,  $n_{\text{NH}_4^+}$  is background at‰  $^{15}\text{N}$  of  $\text{NH}_4^+$ , and  $[\text{NO}_3^- + \text{NO}_2^-]$  is the concentration of the  $\text{NO}_x^-$  pool. Consistent with the results of Kanda et al. (18), we assume that isotope enrichment of the substrate changed negligibly in the open ocean waters we examined. Photoautotrophic uptake of  $^{15}\text{NH}_4^+$  in our samples is likely to be minimal because our samples were collected deep within the euphotic zone (Fig. S2) and were incubated in the dark (main text *Results and Discussion*).

$\text{NH}_4^+$  uptake was not measured, but this would not affect rate calculations because the modest isotopic fractionation associated with this ( $\epsilon \sim -10\text{‰}$ ; ref. 19) would not appreciably change the isotopic composition of the heavily labeled  $\text{NH}_4^+$  pool. Release of  $\text{NH}_4^+$  during the incubation could increase the unlabeled  $\text{NH}_4^+$  pool and therefore affect rate calculations, but this possibility is not supported by our data, either: Total  $[\text{NH}_4^+]$  would have to increase by at least six- to eightfold in every experimental treatment, but not in controls, to dilute the added isotopic label sufficiently to explain the lack of isotopic enrichment seen in the oxidized nitrogen pool.

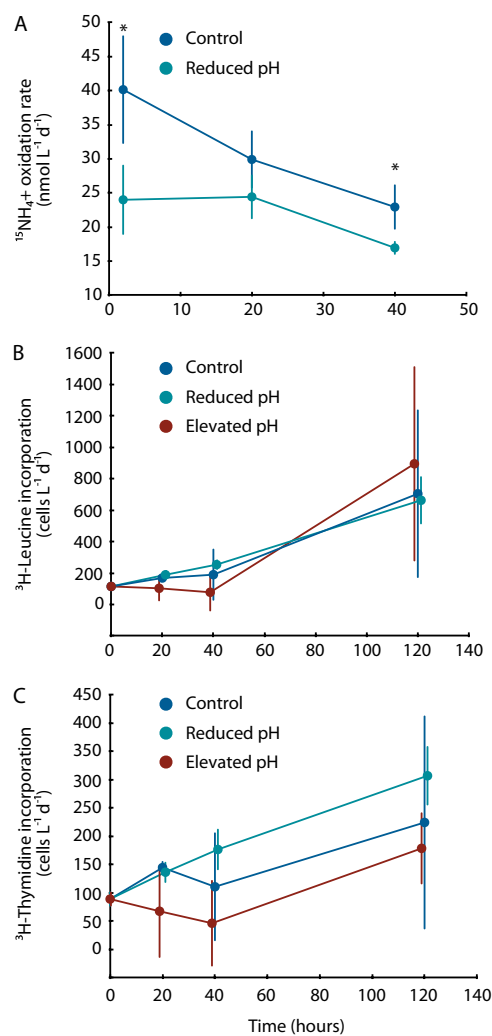
**Additional Calculations.**  $\text{NH}_3$  concentrations were calculated on the basis of  $\text{NH}_4^+$  concentrations, pH, and  $\text{pK}_a$  values using Eq. S2:

$$[\text{NH}_3] = [\text{NH}_4^+] \times 10^{(\text{pH} - \text{pK}_a)}. \quad [\text{S2}]$$

Percentage changes in ammonia oxidation rates with projected changes in ocean pH were determined on the basis of the slope of the relationship between normalized ammonia oxidation rates and  $[\text{H}^+]$ . To normalize ammonia oxidation rates, we divided measured rates by the maximum measured rate in each experiment. Slopes between normalized rates and  $[\text{H}^+]$  were assumed to be linear across pH values from 8.1 to 8 and were multiplied by the overall change in  $[\text{H}^+]$  from pH 8.1 to 8.05 and from pH 8.05 to 8. This calculation yields a range of expected changes across the different experiments, which are shown in Fig. 4. To calculate expected changes in  $\text{N}_2\text{O}$  production, we assumed that half of oceanic  $\text{N}_2\text{O}$  is produced via nitrification (20) and multiplied the oceanic source reported in the most recent Intergovernmental Panel on Climate Change report (21) by the range of percentage changes calculated above.

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**Fig. S3.** Microbial responses to ocean acidification over time during the SPOT experiment, including (A)  $^{15}\text{NH}_4^+$  oxidation rates, (B)  $^3\text{H}$ -leucine incorporation, and (C)  $^3\text{H}$ -thymidine incorporation. Significant differences (ANOVA  $P < 0.05$ ) between treatments are denoted by asterisks. Data points represent the mean of triplicate measurements and are offset in B and C to allow for better comparison; error bars depict 1 SD of triplicate measurements.



**Table S1. Regression statistics for [H<sup>+</sup>], pH, and NH<sub>3</sub> and their relative percentage change, in relation to ammonia oxidation rates and their relative percentage change**

Variable	Correlation with rate	Variable	Correlation with % change in rate
[H <sup>+</sup> ]	0.0002	% change in [H <sup>+</sup> ]	0.8101*
pH	0.0001	% change in pH	0.8275*
[NH <sub>3</sub> ]	0.3371*	% change in [NH <sub>3</sub> ]	0.8463*
[NH <sub>3</sub> ] <sub>Sargasso Sea</sub>	0.8675*		
[NH <sub>3</sub> ] <sub>other experiments</sub>	0.7887*		

\* $P < 0.05$ .

**Table S2. Compilation of pH values and  $^{15}\text{NH}_4^+$  oxidation rates for all experiments and treatments**

Experiment, treatment	pH	$^{15}\text{NH}_4^+$ oxidation rate (nmol·L <sup>-1</sup> ·d <sup>-1</sup> )
Sargasso 1, 2× CO <sub>2</sub>	7.99 (±0.01)	1.02 (±0.10)
Sargasso 1, 1× CO <sub>2</sub>	8.00 (±0.01)	1.01 (±0.48)
Sargasso 2, 2× CO <sub>2</sub>	8.02 (±0.02)	1.29 (±0.45)
Sargasso 2, 1× CO <sub>2</sub>	8.08 (±0.02)	1.37 (±0.35)
Sargasso 2, 0.5× CO <sub>2</sub>	8.09 (±0.01)	1.58 (±0.16)
BATS, control	8.06 (±0.11)	16.7 (±1.9)
BATS, 2× CO <sub>2</sub>	7.93 (±0.12)	10.3 (±0.4)
BATS, 1× CO <sub>2</sub>	7.99 (±0.10)	8.13 (±1.01)
SPOT, control 3 h	n.m.	40.2 (±7.8)
SPOT, 2× CO <sub>2</sub> 3 h	n.m.	24.0 (±5.0)
SPOT, 1× CO <sub>2</sub> 3 h	n.m.	15.5 (±1.8)
SPOT, control 20 h	8.01 (±0.02)	29.9 (±4.1)
SPOT, 2× CO <sub>2</sub> 20 h	7.96 (±0.03)	24.4 (±3.1)
SPOT, 1× CO <sub>2</sub> 20 h	8.05 (±0.01)	18.9 (±3.1)
SPOT, control 40 h	8.00 (±0.02)	22.9 (±3.2)
SPOT, 2× CO <sub>2</sub> 40 h	7.91 (±0.02)	16.9 (±0.9)
SPOT, 1× CO <sub>2</sub> 40 h	8.07 (±0.01)	13.8 (±2.9)
HOT 209, control	n.m.	8.14 (±0.65)
HOT 209, 1× acid	n.m.	7.50 (±0.72)
HOT 209, 2× acid	n.m.	7.40 (±0.20)
HOT 209, 3× acid	n.m.	6.81 (±0.76)
HOT 210, control	7.99	2.52 (±0.10)
HOT 210, 1× acid	7.85	1.61 (±0.23)
HOT 210, 2× acid	7.63	1.77 (±0.22)
HOT 210, 3× acid	7.42	2.55 (±0.55)

Values in parentheses represent SDs of triplicate treatments. n.m., not measured.